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The Sunburn Cell in Hairless Mouse Epidermis: Quantitative Studies with UV-A Radiation and Mono- and Bifunctional Psoralens

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The production of the sunburn cell by UV-A radiation and topical psoralens in hairless mouse epidermis has been studied. It has been shown that the appearance of this cell is dependent on the dose of both UV-A radiation and of the psoralen. The time-course with 8-methoxypsoralen has peak sunburn cell numbers at 28 hr postirradiation. A comparison of 2 bifunctional (8-methoxypsoralen and 5-methoxypsoralen) and 2 monofunctional (angelicin and 3-carbethoxypsoralen) psoralens showed the former are more potent. This suggests that DNA crosslink lesions may play a rôle in sunburn cell production.

The so-called sunburn cell (SBC) with its pyknotic nucleus and eosinophilic cytoplasm is a characteristic histopathological feature of mammalian epidermis after exposure to ultraviolet radiation [1,2] and may be considered as an example of "cell shrinkage necrosis" in the epidermis [3]. UV-C radiation (at 254 nm) and UV-B radiation (280-315 nm) readily provoke the appearance of the SBC [2] but UV-A radiation (315-400 nm) has little or no such effect [4,5]. However, SBCs in both rodent and human epidermis are provoked by combined UV-A radiation and 8-methoxypsoralen (8-MOP) [2,6]. Ultrastructural studies have shown these 8-MOP and UV-A radiation SBCs to be similar to the UV-B radiation SBC in human skin [7]. Figure 1 shows an electron micrograph of a SBC (254 nm induced) in hairless mouse epidermis. Characteristics typical of a human SBC may be observed, viz., perinuclear clumping of tonofilaments, reduced number of desmosomes, perinuclear halo formation and intracellular vacuolization [8].

Psoralens are compounds which, in the presence of UV-A radiation, form mono-adducts and crosslinks with DNA [9,10]. Compounds that form only mono-adducts are termed monofunctional and those that form crosslinks are termed bifunctional. 8-MOP, a bifunctional psoralen, is now routinely used in the photochemotherapy (PUVA) of psoriasis [11]; the supposed rationale of which is the inhibition of epidermal DNA synthesis, although there has been speculation on a photo-immunological aspect in the control of this disorder [12]. Hönigsmann et al [13] have suggested that 5-methoxypsoralen (5-MOP), also a bifunctional psoralen, is superior to 8-MOP, for photochemotherapy. It is generally thought that the crosslink reaction is the more deleterious to the cell, possibly because this lesion is less readily repaired [14]. This has prompted recent interest in the use of monofunctional psoralens, e.g, 3-carbethoxypsoralen (3-CP), in the photochemotherapy of psoriasis [15], the rationale being that these compounds may do less serious damage to DNA and thus reduce the risk of skin cancer that has been reported in some of the patients treated with PUVA [16].

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Abbreviations:
3-CP: 3-carbethoxypsoralen
5-MOP: 5-methoxypsoralen
8-MOP: 8-methoxypsoralen
SBC: sunburn cell

The SBC provides a convenient and objective quantitative measure of acute UVR induced epidermal damage to mammalian epidermis. It has been speculated that it may also give an indication of photochemical damage to epidermal DNA [2,17,18]. The studies described here provide some further quantitative data on SBCs induced by psoralens and compare the effects of 2 bifunctional and 2 monofunctional psoralens.

MATERIALS AND METHODS

Animals

These were male hairless albino mice, 4–6 weeks old; a long established in-bred strain of the Institute of Dermatology, London.

Psoralens

The bifunctional psoralens were 8-MOP and 5-MOP. The monofunctional psoralens were angelicin and 3-CP.

Compounds were dissolved in absolute ethanol (1.85 mM). Purity was verified by TLC, mass spectrometry, and absorption spectroscopy.

Irradiation Sources and Radiometry

The UV-A radiation source was a Waldmann 4000 horizontal PUVA unit with Sylvania FR90T12 fluorescent tubes. The emission spectrum covers the complete UV-A radiation range with a peak in the region of 360 nm and with about 0.5% total output in the UV-B radiation region [19]. Irradiance at the mouse skin surface, usually about 5 $mW.cm^{-2}$, was measured with an International Light IL442A photochemotherapy radiometer calibrated at 365 nm by the National Physical Laboratory (Teddington, U.K.).

Experimental Procedures and Data Analysis

See Young and Magnus [18] for details. In summary, animals were irradiated approximately 1½ hr after topical application of the test solution on flank skin; 50 μl was pipetted over an area 1 × 1 cm. Each experimental point is from 5 to 7 animals; control data are from separate animals. Hematoxylin and eosin stained paraffin sections (8 μm) were examined microscopically and SBCs counted. The main criterion for a SBC was a vacuolated cell with a pyknotic nucleus. The degree of cytoplasmic eosinophilia ranged from very faint to quite marked. SBC counts appeared to follow a log normal rather than a normal distribution, so all statistical analysis has been carried out using the transformation Log_{10} ((no. $SBC.cm^{-1}$ of skin) + 1) to avoid the difficulty of zero counts in control studies. Regression analyses were carried out using all experimental observations rather than mean values.

Results

Figure 2 shows a UV-A radiation dose-response curve, keeping 8-MOP dose constant, at 38 hr postirradiation. This shows a clear log-log linear relationship between UV-A radiation dose and SBC incidence. Figure 3 shows a log-log linear relationship between 8-MOP dose and SBC incidence, also at 38 hr postirradiation. In both these experiments, analysis of variance showed that the regressions were highly significant ($p < 0.005$) and that all variation was due to experimental error rather than lack of fit to a log-log linear model. Figure 4 shows a time-course for UV-A radiation and 8-MOP induced SBC production. Peak numbers are observed at about 28 hr. Note that control treatment, i.e., UV-A radiation plus vehicle, resulted in virtually no SBCs, as did treatment with 8-MOP but no UV-A radiation. Values above the zero baseline are largely the result of the transformation Log_{10} $(x + 1)$. Figure 5 shows the results of treatments with UV-A radiation and 8-MOP, 5-MOP, angelicin and 3-CP. Sacrifice time was 20 hr. Treatment with 3-CP and UV-A radiation had the same effect as control treatments, UV-A radiation with vehicle and psoralens alone, i.e., no effect. The 8-MOP data were fitted to a linear model but it was found that the 5-MOP and angelicin data gave better fits with 2nd and 3rd order polynomial regressions respectively.

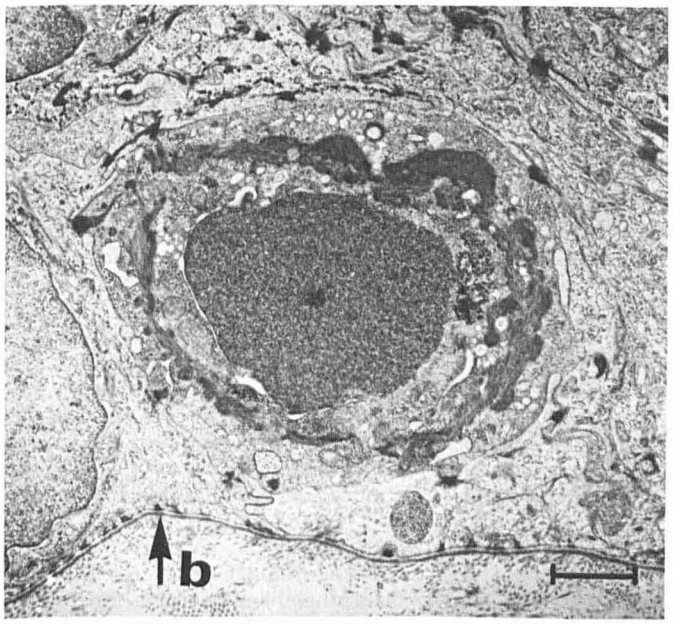

FIG 1. Electron micrograph of SBC (254 nm) in hairless mouse sacrificed 10 hr postirradiation. Note features in common with SBC observed in human epidermis. Condensed nucleus with perinuclear clumping of tonofilaments, loss of desmosomes and intracellular vacuolization, *b* = basal lamina, *scale bar* = 2 μm.

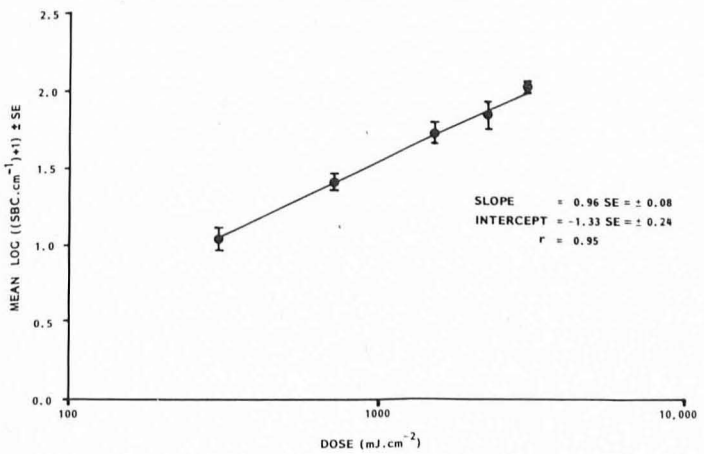

FIG 2. UV-A radiation dose-response curve with 8-MOP at 1.85 mM.

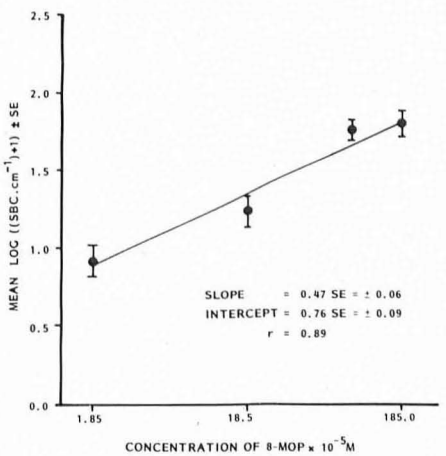

FIG 3. 8-MOP dose-response curve with exposure to 3.3 J.cm^{-2} of UV-A radiation. Note, calculation of regression parameters with M × 10^6.

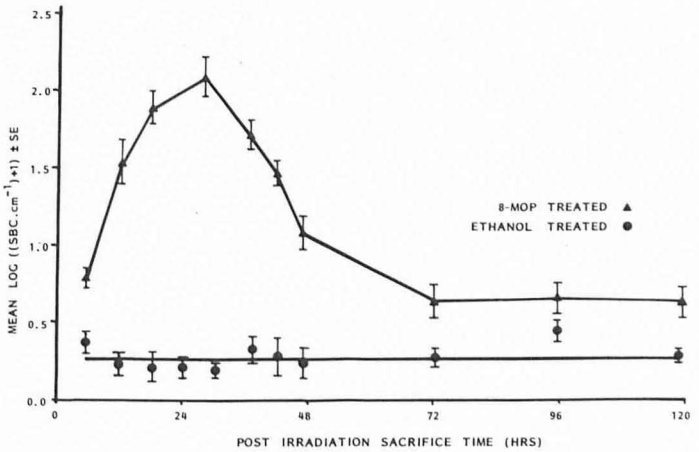

FIG 4. Time-course with 1.8 J.cm^{-2} of UV-A radiation and 8-MOP at 1.85 mM.

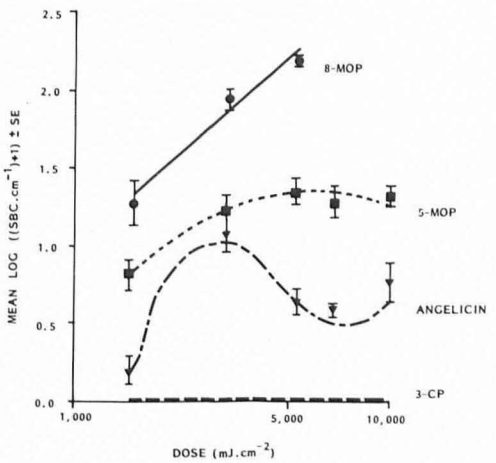

FIG 5. UV-A radiation dose-response studies with 4 psoralens at 1.85 mM .

DISCUSSION

Dose-Response Studies

SBC production by topically applied 8-MOP and 5-MOP at constant dose has been shown to be related to UV-A radiation dose. The UVR dose-response curve obtained with angelicin shown in Figure 5, the result of several experiments, is anomalous. With a constant dose of UV-A radiation an increase of 8-MOP dose also increases SBC numbers. Dose-response data from Figs 2 and 3 may be combined if, at every data point, the products of 8-MOP and UV-A radiation dose are plotted against SBC number. The linear relationship which is then observed (correlation coefficient = 0.92) suggests an inverse relationship between 8-MOP and UV-A radiation dose similar to that observed for the inhibition of DNA synthesis in mammalian cells *in vitro* [20].

Time-Course

The time-course study with 8-MOP and UV-A radiation shows peak SBC numbers at 28 hr. This time for maximum SBC count is similar to that observed for UV-B radiation (24 hr) but much later than that of UV-C radiation (8 hr) [2]. Assuming no major rôle for phagocytosis or other modes of degradation, these times must represent the mean minimum transit times from the primary photochemical event to desquamation. As SBCs were often observed in the basal layer, these values may be indicative of the times taken for migration from the basal layer to the stratum corneum where SBCs were also

observed. By 72 hr, with 8-MOP and UV-A radiation, and in less time with UV-B and UV-C radiation, there is little trace of SBCs in the epidermis. Potten [21] has shown that in the dorsal skin of the haired mouse, minimum transit time from basal layer to top granular layer is 5 days and from the top granular layer to the surface is 6 days, a result he found to be in agreement with those of other workers both in the haired and hairless mouse. Therefore, the SBC would seem to have a rather accelerated passage through the epidermis, suggesting a dynamic process rather than a passive one, or be subject to rapid degradation. Jarrett [22] has commented on the high mobility of keratinocytes *in vitro* and suggested a possible relationship with tonofibril contractile ability. A disturbed tonofibril pattern is an electron microscope characteristic of the SBC, and the associated loss of desmosomes may result in decreased intercellular adhesion.

Hairless mice treated with topical 8-MOP and UV-A radiation showed maximal edema at 24 hr [23]. Similar time-courses for erythema/edema and SBC production in the same species might suggest common underlying mechanisms or a dependence of erythema/edema on UVR damage to epidermal cells. A dependence of SBCs on the erythema reaction seems unlikely as indomethacin does not affect SBC production but inhibits the erythema response [24,25]. The rank order of time-course maxima for erythema in human epidermis induced by UV-C and UV-B radiation and PUVA [26,27] is similar to that of SBC induction in mouse epidermis. However, a study using semi-quantitative methods [28], where UVR doses were matched to give equal degrees of erythema, showed no substantial differences in the time-course patterns for SBCs induced by UV-A, UV-B and UV-C radiation and UV-A radiation with psoralens.

The Role of Crosslinks and Mono-adducts

DNA-psoralen crosslinks have been demonstrated *in vivo* in guinea pig skin [29,30,31] and in hairless mouse skin [32]. Both the bifunctional psoralens, 8-MOP and 5-MOP, readily induce SBCs; per contra angelicin had little effect and 3-CP none at all. These differences in the effects of bifunctional and monofunctional psoralens may suggest a rôle for DNA crosslinks in SBC formation.

DNA photoreactivity *in vitro* with 8-MOP and 5-MOP is about 4 and 2 times greater respectively, than with angelicin [33]. With both psoralen and 8-MOP, most of the DNA lesions formed *in vitro* are mono-adducts rather than crosslinks [34, 35], therefore, it may be misleading to assign an effect to crosslinks when it may be the consequence of a greater number of mono-adducts. However, in the synthetic monofunctional psoralen 3-CP, binding with DNA is substantially higher than that of 8-MOP both *in vitro* and *in vivo* [36] but SBCs were not observed with this compound. In yeast survival studies, Averbeck, Moustacchi, and Bisagni [36] found that 8-MOP was more potent than 3-CP; a result that strongly suggests that crosslinks are more lethal.

3-CP was shown to undergo rapid photodegradation when exposed to the broad band UV-A radiation used in these experiments. The source used by Averbeck, Moustacchi, and Bisagni [36] had a maximum output at 365 nm but no emission below 340 nm so it is possible that photodegradation takes place in preference to DNA photobinding when 3-CP is irradiated with shorter wave UV-A radiation.

At comparable UVR dose points, 8-MOP and 5-MOP were more active than angelicin by factors of 30.6, SD ± 19.3 and 5.6, SD ± 2.7 respectively. Interestingly, Coppey, Averbeck, and Moreno [14] found 8-MOP about 36 times more effective than angelicin in inhibiting colony-forming ability of CV-1 monkey kidney cells in tissue culture.

At comparable UVR dose points, 8-MOP is more potent than 5-MOP by a factor of 5.3, SD ± 0.8. 8-MOP also seems to be more potent than 5-MOP for erythema induction in human [37] and guinea pig [33,38,39] skin. Neither 3-CP nor angelicin readily induce erythema [15,33]. In some micro-organisms, 5-MOP appears to be a more phototoxic agent than 8-MOP. In the yeast *Saccharomyces cerevisiae* Averbeck (personal communication) found the former to be more potent by a factor of 2.5 in survival studies and 3 to 4 times more effective in the induction of "petite mutations." 5-MOP was also more potent than 8-MOP with respect to survival and growth inhibition in *Candida albicans* and cytolysis in ciliates [40].

The fact that differences in effect between these 2 compounds are not consistent and cannot be readily related to their *in vitro* binding with DNA [10,41], suggests that the mechanisms by which they exert their end-points in different systems may not be similar.

The differences in the numbers of SBCs induced by the bifunctional psoralens and angelicin are sufficiently greater than their differences in DNA photoreactivity *in vitro* [33] to imply a qualitative difference between the sunburn cell provoking effects of crosslinks and mono-adducts, thus suggesting that the DNA crosslink is the more significant lesion. The action spectrum for 8-MOP and UV-A radiation induced SBCs has a peak in the 320–335 nm region and is consistent with the hypothesis that DNA crosslink damage provokes the SBC [18]. A similar action spectrum has been reported for 8-MOP-DNA crosslinking *in vitro* [42].

Woodcock and Magnus [2] suggested DNA as a possible chromophore for SBCs induced by UV-B radiation. It has been demonstrated that SBCs are much less likely to show DNA repair as manifest by unscheduled DNA synthesis when compared with normal adjacent keratinocytes [17]. Recent studies by Danno, Takigawa, and Horio [43] also implicate DNA as a possible chromophore. However, to date, all evidence for DNA is circumstantial. The SBC data obtained from the psoralen studies described are still circumstantial with respect to DNA as a target molecule because psoralens also photoreact with proteins [44] and the consequences of these reactions are not known.

A Possible Relationship between SBCs and Skin Cancer

Whether the SBC has any special significance, other than that of a dying cell, is unknown, but it has been described as an example of apoptosis, viz., programmed cell deletion which characteristically affects scattered single cells [45].

Cairns [46] has speculated on the evolution of mechanisms that protect the animal from "fitter," i.e., more prolific cells arising during its lifetime. Danno, Takigawa, and Horio [43] have provided experimental evidence that suggests that proliferative (stem) cells are more prone to becoming SBCs. As a dying cell, the SBC may be presumed to be without neoplastic potential and as such may have a "protective" role if DNA is a chromophore. Both the bifunctional psoralens, 8-MOP and 5-MOP, are photocarcinogenic in mice [47,48]. If, as speculated, crosslinks are largely responsible for the SBC, it may be the mono-functional lesions, produced in much greater number and much more readily repaired [14] with the possibility of error, that give rise to tumors. 3-CP was shown not to induce the SBC but is also reported as nonphotocarcinogenic [15]. As already described, this psoralen is very photolabile and so may not be the best monofunctional compound for such studies.

If a relationship between psoralen SBC production and skin cancer in animals could be demonstrated this might be useful in assessing the risk of photocarcinogenesis by psoralens in humans.

We thank Mr. T. Cowen for the electron micrograph as shown in Figure 1.

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